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Authors: Juchno, Dorota, and Boroń, Alicja

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Fecundity of the spined loach, *Cobitis taenia* (Pisces, Cobitidae) and natural allopolyploids of *Cobitis* from a diploid-polyploid population

Dorota JUCHNO and Alicja BOROŃ

Department of Zoology, University of Warmia and Mazury, Oczapowski St. 5, 10-957 Olsztyn, Poland; e-mail: juchno@uwm.edu.pl

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Abstract. A comparison was made between the absolute and actual fecundity of *C. taenia* and allotriploid *Cobitis* females from a diploid-polyploid population inhabiting the Bug River, of Vistula River basin in Poland. All specimens were measured, weighed and aged. The absolute fecundity was determined by a gravimetric method using 29 ovaries (three of *C. taenia*, 25 of triploids and one of tetraploid). The actual fecundity was estimated according to the method adopted by Halačka et al. (2000) and used five ovaries of *C. taenia* and 40 of *Cobitis* triploids. Absolute fecundity of *C. taenia* under analysis ranged from 1 819 to 3 302 eggs and equaled 2 487 on average. It was significantly lower than absolute fecundity of an exclusively diploid population in Klawój Lake described previously (Juchno & Boroń 2006a). Absolute fecundity of triploid females ranged from 285 to 3 710, with an average of 1 577 eggs, whereas the fecundity of tetraploid *Cobitis* female was low, with only 882 eggs. The highest actual fecundity of *C. taenia* as well as of triploids was observed after they laid their first batch of eggs.

Key words: triploids, absolute fecundity, actual fecundity, reproduction

Introduction

Populations composed exclusively of *Cobitis taenia* L. diploids are rare in Poland. As is commonly found, polyploid hybrids are formed with the Danubian spined loach *C. elongatoides* Bacescu & Maier and/or *C. tanaitica* Bacescu & Maier and thus mixed diploid-polyploid populations appear (Boroń 1999, 2003). In mixed populations triploid females are the most common, accounting for about 95% of all catches of *Cobitis* (Boroń 2003). This dominance may be influenced by gynogenetic reproduction (Vasil'ev et al. 1989, Saat 1991), a longer spawning period or laying bigger eggs in comparison with diploids (Juchno et al. 2007). Relatively small numbers (about 3%) of allotetraploids in mixed *Cobitis* populations have resulted perhaps from reproductive limitations caused by abnormal structure of gonads (Vasil'ev et al. 2003, Juchno & Boroń 2006b, Juchno et al. 2007).

The fact that the fishes with different ploidy are morphologically indistinguishable makes examination very difficult. A distinguishing feature of the *C. taenia* is the chromosome number 2n = 48 (Vasil'ev et al. 1989, Boroń 1999).

The loach spawns in batches from May to July (Robotham 1981, Marconato & Rasotto 1989, Boroń & Pimpicka 2000, Juchno & Boroń 2006a, Juchno et al. 2007). For a species like the loach (with its asynchronous development of oocytes and the batch spawning), estimating the absolute fecundity (the number of eggs which can be laid during the given reproductive season), and separating them from the reserve of germ cells (those that stay in the ovary after the reproduction) is difficult and still discussed (Wootton 1979, Halačka et al. 2000, Juchno & Boroń 2006a). The term 'actual fecundity' was proposed by Halačka et al. (2000) in order to give the dynamics of the number of laid eggs. This involved measuring the number of oocytes which potentially could be spawned in a year, by observing them over the whole reproductive season.

The aim of this study was to determine the fecundity of karyologically verified *C. taenia* and allopolyploid females of *Cobitis* from the mixed diploid-polyploid population in the Vistula River basin. The results were compared with the fecundity of *C. taenia* from the exclusively diploid population (Juchno & Boroń 2006a).

Material and Methods

The females of *Cobitis* were collected from a diploid-polyploid population in the Bug River (52º N; 23º30´ E), using a fry trawl with a 2 mm mesh. The fish were transported alive to the Cytogenetic Laboratory in the Department of Zoology and then were karyotyped. The chromosomal preparations were made by the 'splash' technique (Boroń 1999). The fish were measured (SL – body length, with 0.1 mm accuracy) and weighed (Wo - gutted weight, with 0.1 g accuracy). Age was assessed based on the pattern of annual growth of otholits (Boroń et al. 2008).

The ovaries were fixed in 4% buffered formalin. The absolute fecundity (Fa) was determined in three ovaries of *C. taenia* (2n = 48), 25 of triploids (3n = 74) and one ovary of tetraploid female (4n = 98), captured on 19 May 2001 and on 28 April 2002. Among karyologically examined loaches collected in 2001 and in 2002 there were two individuals of *C. taenia* and 19 triploid females, and one *C. taenia* and six triploids, respectively. Absolute fecundity of triploid females captured in the first and second years of the study was statistically different (*t* – test, P = 0.0101) so they were treated separately, whereas Fa of all three *C. taenia* females was counted together. The histological analysis confirmed that the females were in the prespawning period; the ovaries were in the III or IV stages of maturity (Juchno et al. 2007). The absolute fecundity (Fa) was estimated by a gravimetric method (Bagenal & Braum 1978). The oocytes of a diameter over 0.25 mm have been counted using stereomicroscope with an ocular micrometer in a sub-samples of known weight and then the number of all oocytes was counted according to the weight of whole ovaries. Former results obtained by the histological analysis of the ovaries (Juchno et al. 2007) specified that oocytes of 0.25 mm in diameter (in the cortical alveolus stage) can be laid during a given reproductive season. The relative fecundity (Fr) (the number of eggs per female unit weight) was estimated by dividing the absolute fecundity (Fa) by the gutted weight (Wo).

The actual fecundity (F) was estimated according to the method used by Halačka et al. (2000). For calculation five ovaries of *C. taenia* and 40 of triploids, captured during the reproductive season, i.e. on 19 May, 19 June, 10 July and 28 August 2001 have been used. For this purpose either the endogenous and exogenous vitellogenic or the exogenous vitellogenic oocytes, which filled ovaries during the whole reproductive season, were counted. Also a coefficient of variation of the actual fecundity (V) was calculated, according to the equation: V = SD/mean (%).

The relationship between the absolute fecundity (Fa) and selected individual features of the triploid females in the first year of the study (body length and body and gonad weight) was analyzed by using Pearson's correlation coefficient r ($P < 0.05$) and regression equations. Because of relatively scanty material (three individuals), similar correlation for *C. taenia* from the Bug River was not determined. Moreover, statistic comparison between fecundity of *C. taenia* and triploids was not performed.

C. taenia is a protected species in Poland. Hence a special permit had to be obtained from the Minister of the Environment in order to conduct the research (DLOPiKog. - 4201 - 04 - 43/01). Consent for the study was obtained from the local Ethics Commission; individual consent no. 20/01.

Results

Absolute (Fa) and relative (Fr) fecundity

Females of *C. taenia* were 3+ years old. Individual absolute fecundity (Fa) of these fish ranged from 1 819 to 3 302 eggs and equaled 2 487 on average. Relative fecundity (Fr) of *C. taenia* females ranged from 630 to 905, with an average of 811 (Table 1).

Triploid females were divided into classes of 2+, 3+ or 4+ years old (Table 1). The range of Fa varied from 285 to 2 188 eggs in 2001 and from 1 718 to 3 710 in 2002. The average value Fa for all the triploid females was 1 577 eggs. Absolute fecundity of females captured in the first and second years of the study was statistically different (P = 0.0101), therefore in further analyses these specimens were treated separately.

Relative fecundity of 3n females captured in 2001 and 2002 ranged from 123 to 561 and from 481 to 1 288 eggs, respectively (Table 1). The average Fr of triploids was 485 eggs. In both years, the relative fecundity showed a slight increase in proportion with body length, age and weight. However the Fr values of the oldest (4+) and the biggest (> 90,0 mm) females were clearly reduced (Table 1).

Table 1. Absolute (Fa) and relative (Fr) fecundity in particular ranges of body length (SL), body weight (Wo) and age of *C. taenia* and triploid *Cobitis* females from the Bug River. n – number of specimens, SD – standard deviation.

	range	n	Fa range	Fa mean ± SD	Fr range	Fr mean ± SD
			C. taenia (2n)			
SL (mm)	70,1 - 80,0	2	1819 - 2340	2079 ± 368	630 - 897	764 ± 189
	80,1 - 90,0	1	3302	-	905	-
Wo (g)	2,1 - 3,0	2	1819 - 2340	2079 ± 368	630 - 897	764 ± 189
	3,1 - 4,0	1	3302	-	905	-
age	3+	3	1819 - 3302	2487 ± 753	630 - 905	811 ± 156
			Triploid *Cobitis* (3n)			
2001						
SL (mm)	60,0 - 70,0	1	285	-	123	-
	70,1 - 80,0	9	466 - 1391	1119 ± 429	159 - 561	419 ± 141
	80,1 - 90,0	7	1137 - 2128	1681 ± 334	310 - 552	441 ± 103
	>90,0	2	1019 - 2188	1603 ± 826	186 - 450	318 ± 187
Wo (g)	2,1 - 3,0	9	285 - 1502	948 ± 396	123 - 561	375 ± 155
	3,1 - 4,0	5	1137 - 1842	1668 ± 302	338 - 549	497 ± 90
	4,1 - 5,0	4	1345 - 2188	1858 ± 388	310 - 450	395 ± 68
	>5,0	1	1019	-	186	-
age	2+	7	285 - 1502	1015 ± 438	123 - 561	407 ± 165
	3+	7	466 - 1842	1396 ± 546	159 - 549	430 ± 148
	4+	5	1019 - 2188	1691 ± 504	186 - 450	353 ± 110
2002						
SL (mm)	60,0 - 70,0	1	1718	-	1288	-
	70,1 - 80,0	1	1885	-	576	-
	80,1 - 90,0	4	1926 - 3710	2622 ± 767	481 - 1031	659 ± 259
Wo (g)	1,0 - 2,0	1	1718	-	1288	-
	3,1 - 4,0	4	1885 - 3710	2512 ± 851	487 - 1031	683 ± 241
	4,1 - 5,0	1	2323	-	481	-
age	2+	1	1718	-	1288	-
	3+	2	1885 - 1926	1906 ± 29	487 - 576	531 ± 63
	4+	3	2323 - 3710	2853 ± 749	481 - 1031	716 ± 284

A single tetraploid *Cobitis* female was found to have an Fa of 882 oocytes, among which 173 were exogenous vitellogenic oocytes. The absolute fecundity (Fa) and some other analyzed individual features were significantly different among triploid females collected in 2001. The value of the r coefficients was 0.53 for relationships between the Fa - Wo as well as Fa - SL. The highest value of the r coefficient was 0.79 and was found in the relationship between Fa and gonad weight (Wg).

The relationship between Fa and significantly different individual features (Fa - SL, Fa - Wo, Fa - Wg) of triploid females was calculated by logarithmic regression equations (Fig. 1). The lowest value of absolute fecundity (from 285 to 1 500) was seen in the smallest (below 80 mm SL and below 3 g Wo) triploid *Cobitis* females. A distinct increase in Fa value was observed in females with body lengths above 90 mm and weight (Wo) above 4 g.

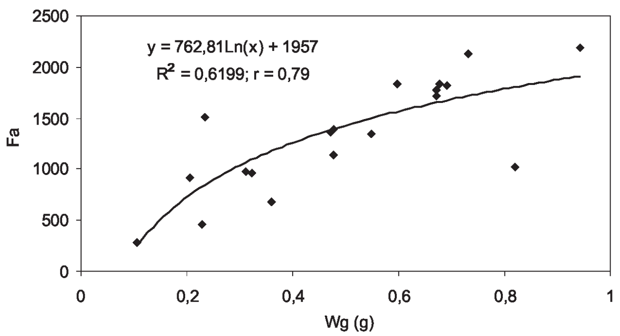

Fig. 1. Relationship between absolute fecundity (Fa) and ovary weight (Wg) of triploid *Cobitis* females from the Bug River in seasonal reproduction 2001, with a regression equation. r – correlation coefficient, R^2 – determination coefficient.

Actual fecundity (F)

The mean actual fecundity (F) of diploid females in May was 2 079, including 790 exogenous vitellogenic oocytes. The ovary weight of diploids (loach) and allotriploids was similar (P = 0.7612). The actual fecundity (F) of triploid females was 1 333, including only 392 exogenous vitellogenic oocytes (Table 2).

In June the actual fecundity of both diploids and triploids reached their highest values of 2 479 and 1 493, respectively. However they were difficult to compare because only one diploid female was collected. The level of actual fecundity differed considerably among triploid females. In the ovaries of some specimens between 251 and 860 oocytes were counted, whereas in the case of one female 4 452 oocytes were found. Similarly the number of exogenous vitellogenic oocytes varied greatly (82.2%) and ranged from 193 to 1 869 (Table 2).

The number of vitellogenic oocytes of one diploid *C. taenia* captured in July was clearly lower than those in June (Table 2). However, triploid females captured in that time were found to have a slight decrease in the mean value of their actual fecundity. Apart from the actual fecundity high inter-individual differences in the ovary weight were observed (Table 2).

After the spawning season had finished, near the end of August, the actual fecundity of both the diploid female and the triploid females achieved its lowest value. Among triploids distinct differences were observed. In some females the actual fecundity was low and amounted to about 400 oocytes, including only about 70 exogenous vitellogenic oocytes (these females had small gonads). In the same time the actual fecundity of other triploids was relatively high and amounted to about 1 500 oocytes, including about 380 exogenous vitellogenic oocytes (these fish had also greater ovary weight).

Discussion

The fecundity of the species under study varies in relation to the size or age of the specimen. Fluctuations in the fecundity of females of similar age or size are due to inter-individual or inter-populational factors and may vary in different years. In a batch spawning species with asynchronous development of oocytes, determination of fecundity is difficult and has been discussed (Baruš & Prokeš 1996, Bohlen 1999, Halačka et al. 2000, Oliva-Paterna et al. 2002, Juchno & Boroń 2006a). The presented data on *Cobitis* fecundity may not be easily compared with the data for other taxa of the

Table 2. Actual fecundity of *C. taenia* (2n) and triploid *Cobitis* (3n) females from the Bug River in seasonal reproduction 2001. SL – standard body length, Wo – body weight, Wg – ovary weight, SD – standard deviation, V – coefficient of variation, n – number of specimens.

date		May		June		July		August	
ploidy		2n	3n	2n	3n	2n	3n	2n	3n
n		2	19	1	10	1	6	1	5
SL	mean	74,6	79,5	-	79,5	-	86,8	-	82,8
(mm)	Min-Max	74,4 – 74,8	70,4–93,4	72,8	71,3–98,2	82,0	73,8–101,5	67,7	78,0–86,6
Wo	mean	2,7	3,4	-	3,5	-	4,0	-	4,0
(g)	Min-Max	2,6 – 2,9	2,2 – 5,5	2,6	2,3 – 6,1	3,4	1,0 – 7,0	2,5	3,1 – 4,9
Wg	mean	0,6	0,5	-	0,7	-	0,7	-	0,3
(g)	Min-Max	0,4 – 0,7	0,1 – 0,9	0,5	0,2 – 1,6	0,4	0,2 – 1,4	0,1	0,2 – 0,5
actual fecundity									
vitellogenic oocytes	mean	2079	1333	-	1493	-	1225	-	876
	Min-Max	1819-2340	285-2188	2479	251-4452	933	367 – 2015	328	366–1494
	SD	368,40	544,13	-	1146,01	-	710,63	-	564,67
	V (%)	17,72	40,82	-	76,76	-	58,01	-	64,46
exogenous vitellogenic oocytes	mean	790	392	-	590	-	511	-	207
	Min-Max	680 - 900	105 - 630	785	193 - 1869	441	159 - 780	167	69 - 381
	SD	155,56	215,99	-	484,91	-	265,05	-	111,92
	V (%)	19,69	55,09	-	82,18	-	51,87	-	54,07

Table 3. Absolute (Fa) and relative fecundity (Fr) in different European populations of *Cobitis*.

species	locality	Fa Range mean	Fr Range mean	references
C. taenia	stream Timonchio, Italy	1012 -	-	Marconato & Rasotto 1989
*C. taenia**	Lake Dgał, Poland	175 – 452 -	-	Białokoz 1986
*C. taenia**	Zegrzynski Reservoir, Poland	112 - 1520 -	28 – 204 108	Boroń & Pimpicka 2000
*C. taenia**	Lake Lucien, Poland	418 - 6800 2180	-	Kostrzewa et al. 2003
C. taenia	Lake Klawój, Poland	869 - 3371 2078	254 - 1062 629	Juchno & Boroń 2006
C. taenia	Bug River, Poland	1819 – 3302 2487	630 – 905 811	Present study
Cobitis (3n)	Bug River, Poland	285 – 3710 1577	123 – 1288 485	Present study
Cobitis (4n)	Bug River, Poland	- 882	- 316	Present study
C. elongatoides	River Danube, Hungary	50 – 1340 -	35 – 110 -	Erős 2000
C. simplicispina	Kızılırmak basin, Turkey	320-2140 -	-	Ekmekçi & Erk'akan 2003
C. paludica	Guadalquivir River, Spain	- 1986	-	Oliva-Paterna et al. 2002
C. paludica	Guadalquivir River, Spain	281 - 1397 1235	-	Soriguer et al. 2000

* no information on the ploidy level

same genus because of various applied methods and lack of information about the ploidy level of examined fishes.

The absolute fecundity of *C. taenia* from the Bug River (Fa = 2 487) under present study was similar as that (Fa = 2 078) of the same species in an exclusively diploid population from Lake Klawój (Juchno & Boroń 2006a). The available data on the absolute and relative fecundities of *Cobitis* in different European populations is shown in Table 3.

The absolute fecundity of the related *Cobitis simplicispina* Hankó from Turkey ranged from 320 to 2 141 eggs (Ekmekçi & Erk'akan 2003) but the values from two populations of *C. paludica* (de Buen) were estimated at 1 986 (Oliva-Paterna et al. 2002) and 1 235 eggs (Soriguer et al. 2000).

A significant correlation between the fecundity, the size of eggs and the size of *C. paludica* females has been observed (Oliva-Paterna et al. 2002). The small females, living in seasonal streams, should maximize the number of eggs they produce as their fecundity is relatively low, whereas bigger females may be able to sacrifice some fecundity in favour of increasing the size of their eggs, which may produce an increase in quality. The maximization of maternal fitness by producing fewer but bigger eggs is likely to be a major influence in survival rates of offspring (Oliva-Paterna et al. 2002).

The published data on fecundity of naturally occurred *Cobitis* polyploids are scarce. The data describe the absolute fecundity of females from mixed *Cobitis* populations, so apart from triploids, it also includes *C. taenia*. The reported level of Fa depended on the eggs' size being taken into account. The absolute fecundity counted by the number of the biggest oocytes (above 0.6 mm or above 1 mm diameter) present in an ovary, amounted to: 175 - 452 of females from Lake Dgał (Białokoz 1986), 208 - 975 of loaches from Lake Lucien (Kostrzewa et al. 2003) and 1 012 of those from north Italy (Marconato & Rasotto 1989). The oocytes counted appeared to have come from the first spawned batches.

A higher level of absolute fecundity based on counting all the vitellogenic oocytes was reported. Boroń & Pimpicka (2000) calculated Fa of loaches of different ploidy levels from the Zegrzynski Reservoir (taking into account oocytes with diameters of over 0.3 mm) as being between 112 and 1 520 eggs. Otherwise absolute fecundity of *Cobitis* females (with an unknown ploidy level) from Lake Lucien (in central Poland) was estimated as an average of 2 180 eggs. The range of Fa was definitely higher, from 418 to 6 800 eggs, due to counting oocytes above 0.2 mm in diameter of both *C. taenia* and polyploid females (Kostrzewa et al. 2003).

Taking into account oocytes with diameters over 0.25 mm, the absolute fecundity of studied triploid *Cobitis* ranged from 285 to 3 710, with an average of 1 577. A significant relationship was found between the absolute fecundity and body size. The smallest females had the lowest level of fecundity (285 eggs). The highest Fa (2 188 eggs) characterized triploid females with body length above 90 mm. The fecundity of one tetraploid *Cobitis* female was low, only 882 eggs, a number probably connected with its gonad histology. In ovaries, amongst germ cells, many somatic cells have been observed (Juchno et al. 2007).

The relative fecundity (Fr) of *C. taenia* from an exclusively diploid population in Lake Klawój was 629 eggs (Juchno & Boroń 2006a) whereas the Fr of those from a diploid-polyploid population in the Bug River was higher (811 eggs). The Fr of *C. elongatoides* was relatively low and ranged from 35 to 110 eggs (Erős 2000), but the Fr of *C. paludica* from Spain decreased during the spawn, from April to July (Oliva-Paterna et al. 2002). This may show a lack of supplementing oocytes from the reserve of previtellogenic oocytes.

Relative fecundity of *Cobitis* from mixed population in Zegrzynski Reservoir ranged from 28 to 204 eggs, with an average of 108 (Boroń & Pimpicka 2000). It was lower than in triploid females from the same type of population in this study, which equaled 485 eggs.

The results obtained seem to support the opinion that calculating the Fa as the number of vitellogenic oocytes in the ovary before the beginning of the spawning (in females spawning in batches with oocytes developing asynchronously) may not be accurate (Mills et al. 1983, Halačka et al. 2000). During spawning season, environmental conditions as well as internal factors (pace of vitellogenesis adjusted hormone, individual changeability), may influence the number of eggs in batches and the number of batches in the reproductive season. It appears that fecundity of *C. elongatoides* females from the Czech Republic increased gradually since April achieving its maximum only in July after laying the first batch. There was an 80% difference in the number of vitellogenic oocytes between April and July (Halačka et al. 2000). This fecundity was determined as the actual fecundity (Halačka et al. 2000).

The value of actual fecundity (F) allows to determine the dynamism of changes in the ovary during the reproductive season, both of all vitellogenic oocytes and of exogenous vitellogenic oocytes which could potentially be the first batch of eggs.

In all diploid and triploid loaches from the Bug River, after autumn - winter resting, the number of oocytes ready to be laid appears to gradually increase, reaching their maximum in June. The highest actual fecundity of both *C. taenia* and triploid females from the Bug River occurred after the first batch (Juchno et al. 2007). *C. taenia* from exclusively diploid population shows the highest fecundity before the spawning period (Juchno & Boroń 2006a). In order to produce and sustain a high number of vitellogenic oocytes, it is necessary to draw on the reserve of previtellogenic oocytes. Similar situations in other batch spawning fish were reported earlier by Mills et al. (1983) and Halačka et al. (2000).

In the loaches from the Bug River, relatively large fluctuations in actual fecundity were recorded, when only exogenous vitellogenic oocytes were counted in an ovary. At the onset of the spawning period, the number of exogenous vitellogenic oocytes ranged from 680 to 900 in diploids and between 193 and 1 869 in triploids. However in loaches from the compared diploid population (in Lake Klawój) the F value ranged from 171 to 1 146 (unpublished data) and seemed to be influenced by high individual variability. The difference between the absolute fecundity of triploid and diploid females probably resulted from differences in the size of oocytes, particularly in the exogenous vitellogenic stage. The triploid females formed significantly bigger oocytes than diploids (Juchno et al. 2007).

Triploid females under present study exhibited a significantly lower absolute fecundity in comparison with that of *C. taenia* (diploids) from Lake Klawój (*t* – test, P = 0.000684). However, both these groups of females were characterized by different size of oocytes in the ovaries. However, oocytes of triploid females were significantly larger than those in diploids (Juchno et al. 2007). This indicates that triploids produce not more but larger eggs, and thus have potentially better adapted fry with a better chance of survival in changing environmental conditions (Juchno et al. 2007). Fluctuations in fecundity may reflect the difference in size of eggs. The maximum size of an egg will be determined by the amount of space needed by larvae to reach their optimum size (Wootton 1979, Kjesbu et al. 1998). There is potential conflict between producing a larger number of smaller eggs in order to offset high hatch mortality, and producing a small number of larger eggs to ensure the offspring's better individual condition (Saat et al. 2003). The obtained results suggest that the fecundity level is individually dependent and is highly variable among triploid females.

Conclusions

The presented results revealed that the estimation of absolute fecundity of species that spawn in batches such as diploid and polyploid *Cobitis* females is complicated. The absolute fecundity calculated as the number of eggs which could be laid during the given reproductive season is very variable among individuals. In order to show the actual number of produced eggs, a calculation is necessary for absolute fecundity for every year of life ('life fecundity'), even in short-lived species.

The life expectancy of triploid *Cobitis* females from the mixed population in the Bug River is one year longer than that of *C. taenia* females from the same population (Boroń et al. 2008). Possibly, the "life fecundity" of triploids is higher than that of *C. taenia* diploids and could be one of the possible explanations for their domination in the *Cobitis* population.

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